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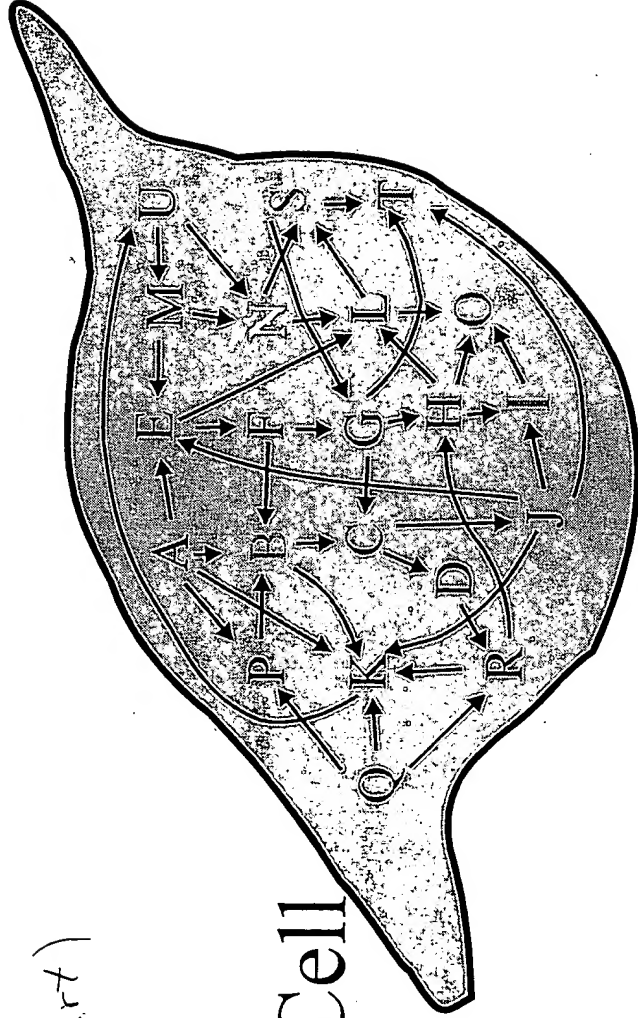
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Fig. 1

(prior art)

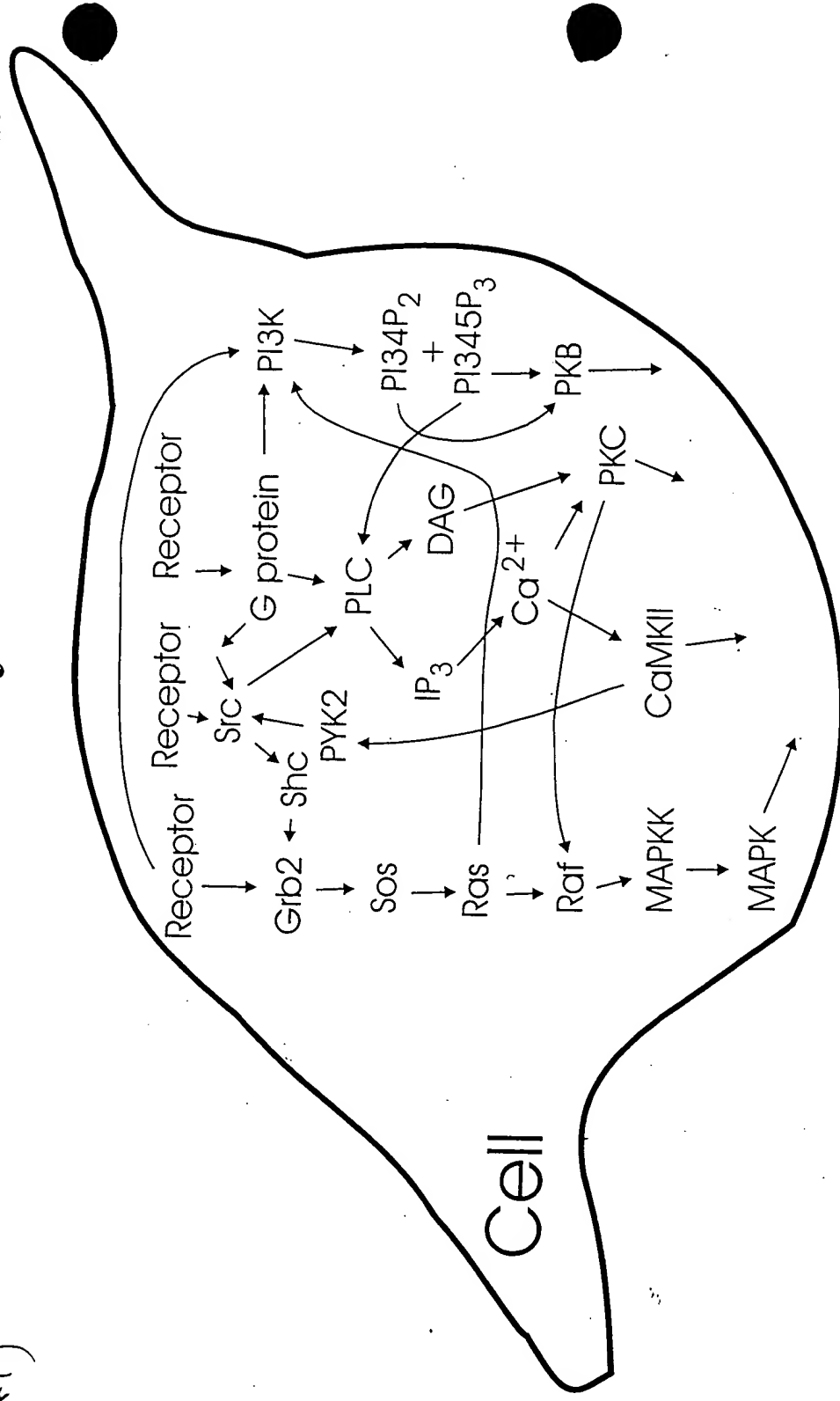
Cell



**Fig. 2**

(prior art)

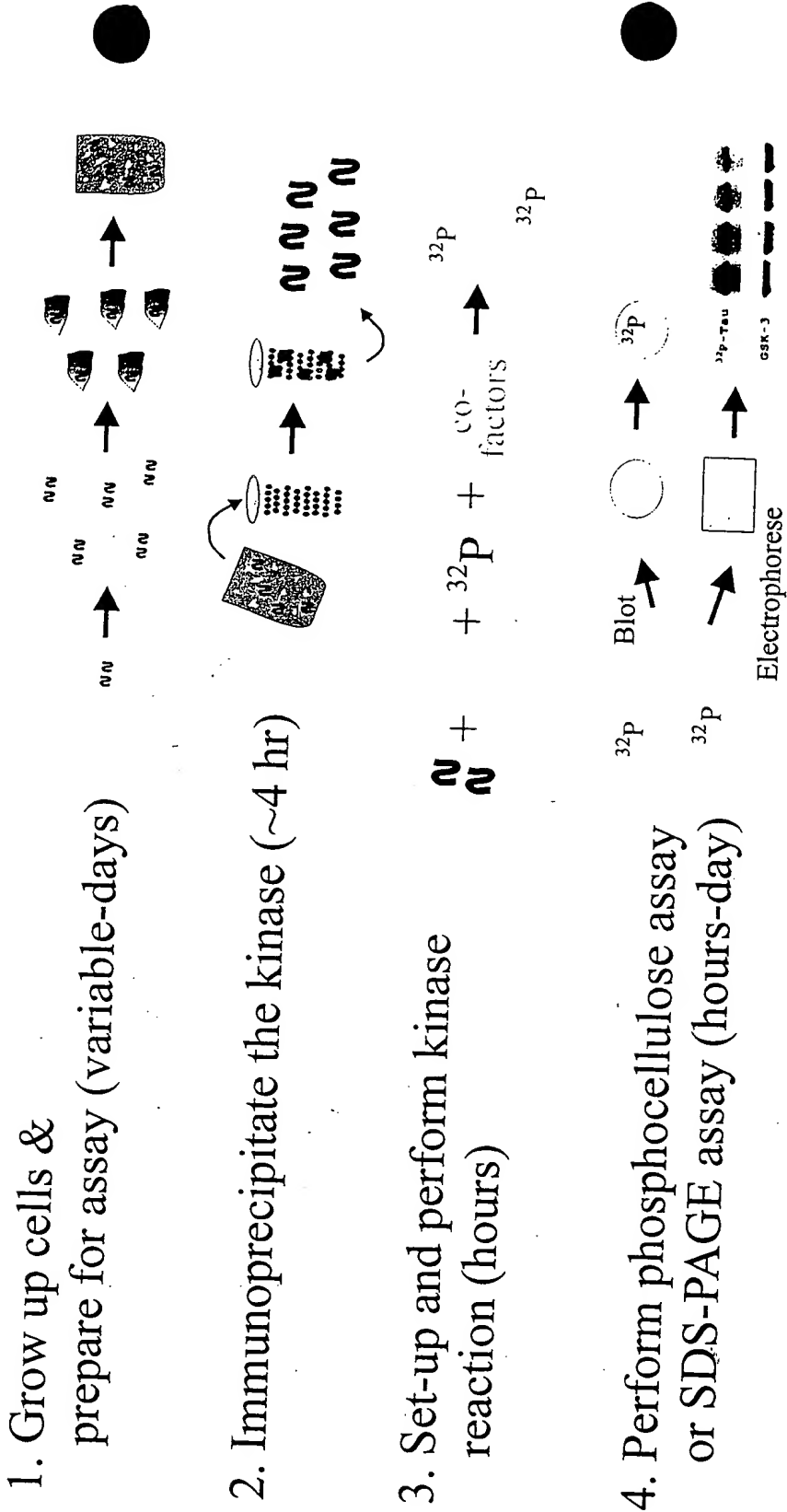
## MAP Kinase/Phosphoinositide/ PI3 Kinase Pathways in the Network



# Fig. 3

(prior art)

## Measurement of Kinase Activation (Current Technology)

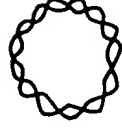
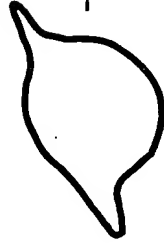


# Fig. 4

(prior art)

## Measurement of Protein Location (via GFP Tag)

1. Develop stably transfected cell lines carrying the overexpressed GFP-tagged protein



2. Fluorescent imaging and pattern recognition

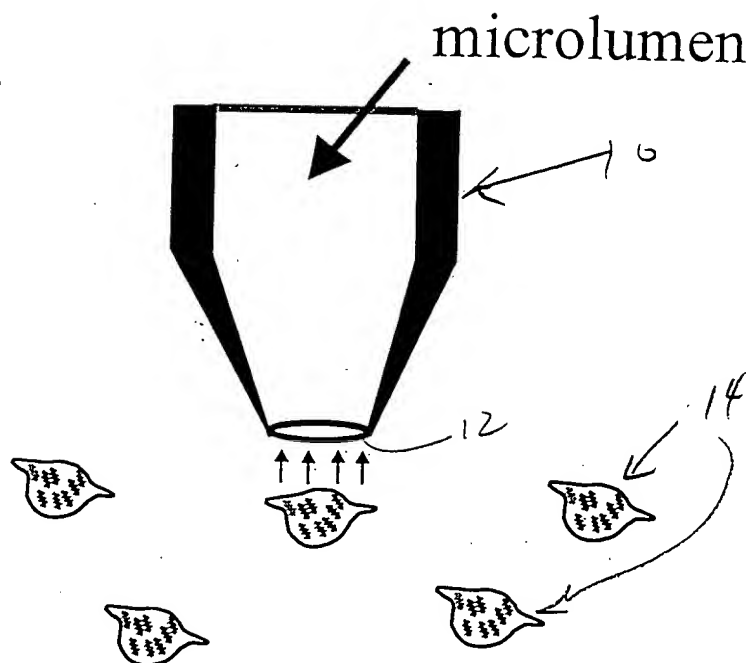


3. Infer protein activity from location

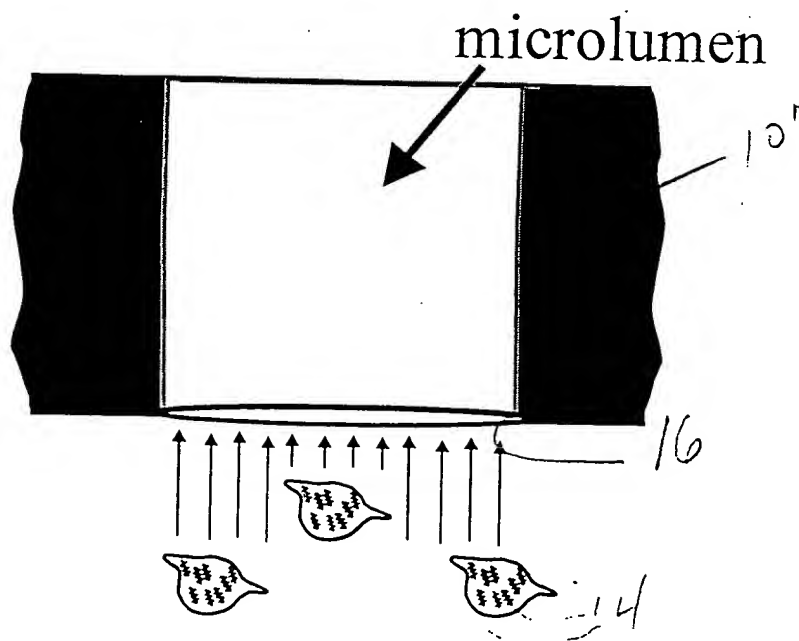
Assay Time ~Minutes

**Fig. 5A**

Single

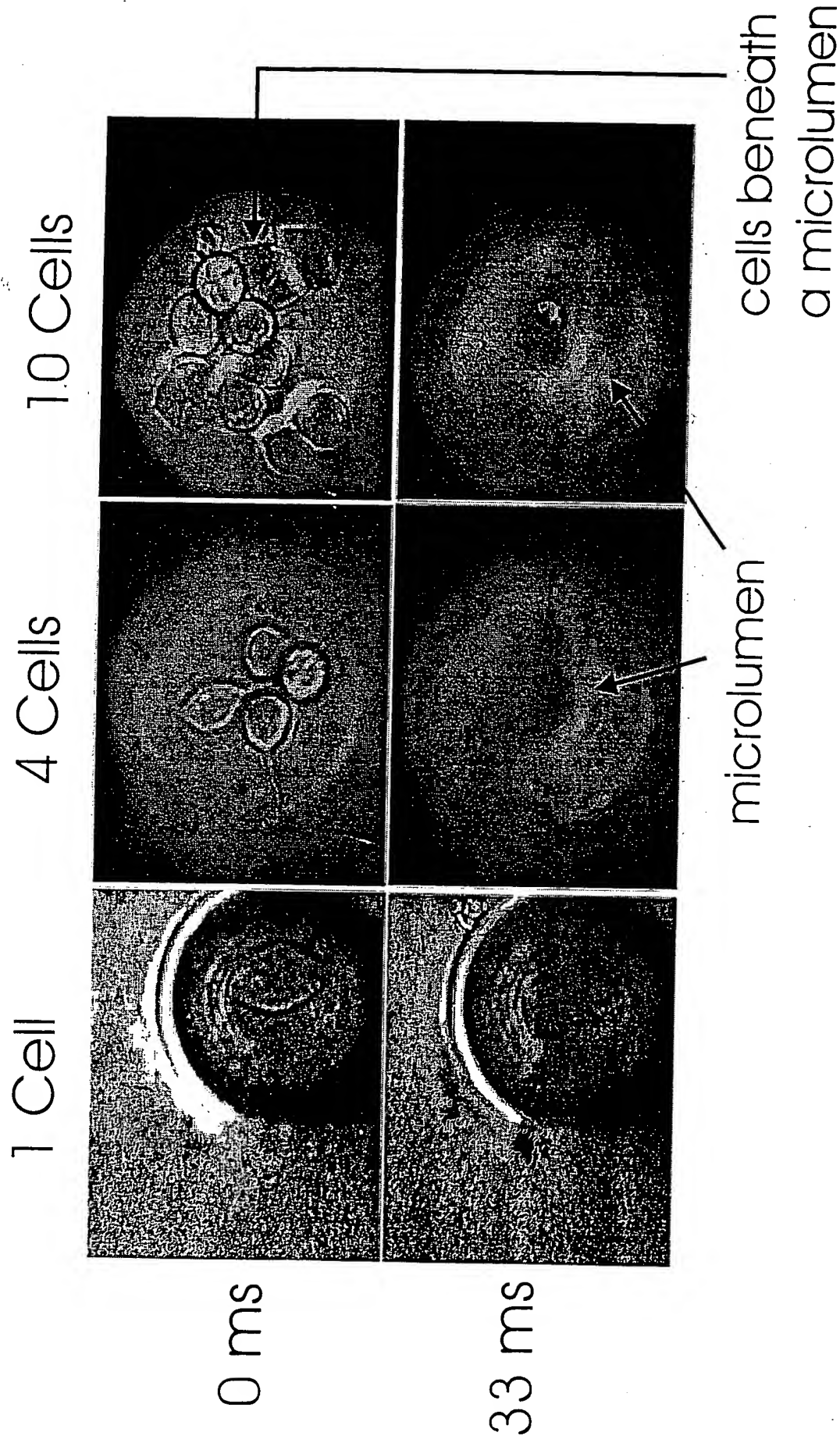


Multiple



**Fig. 5B**

# Performing "Population Average" Measurements



# Single Cells or Population Averages

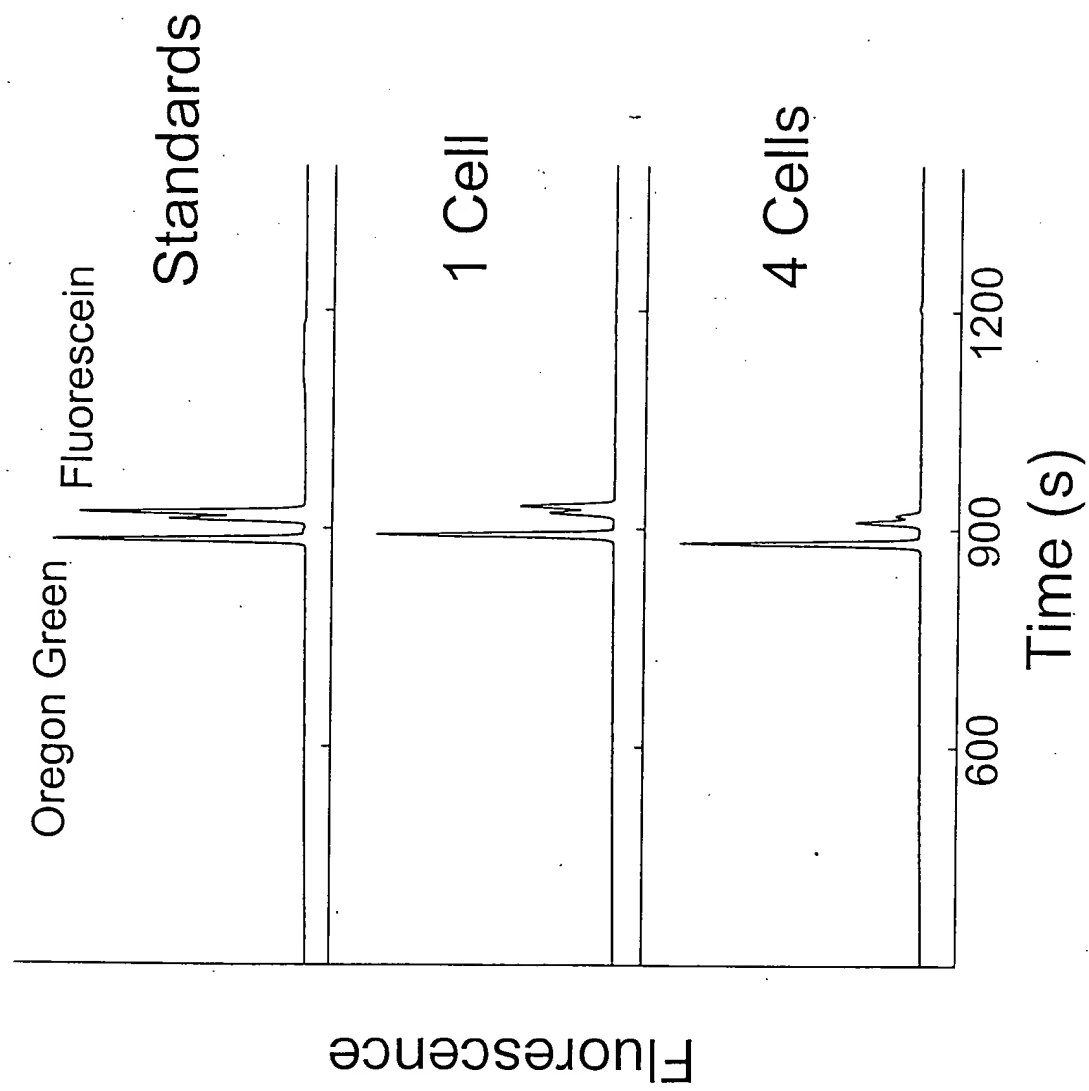


Fig. 5C



# Fig. 6A Sampling a Portion of a Cell

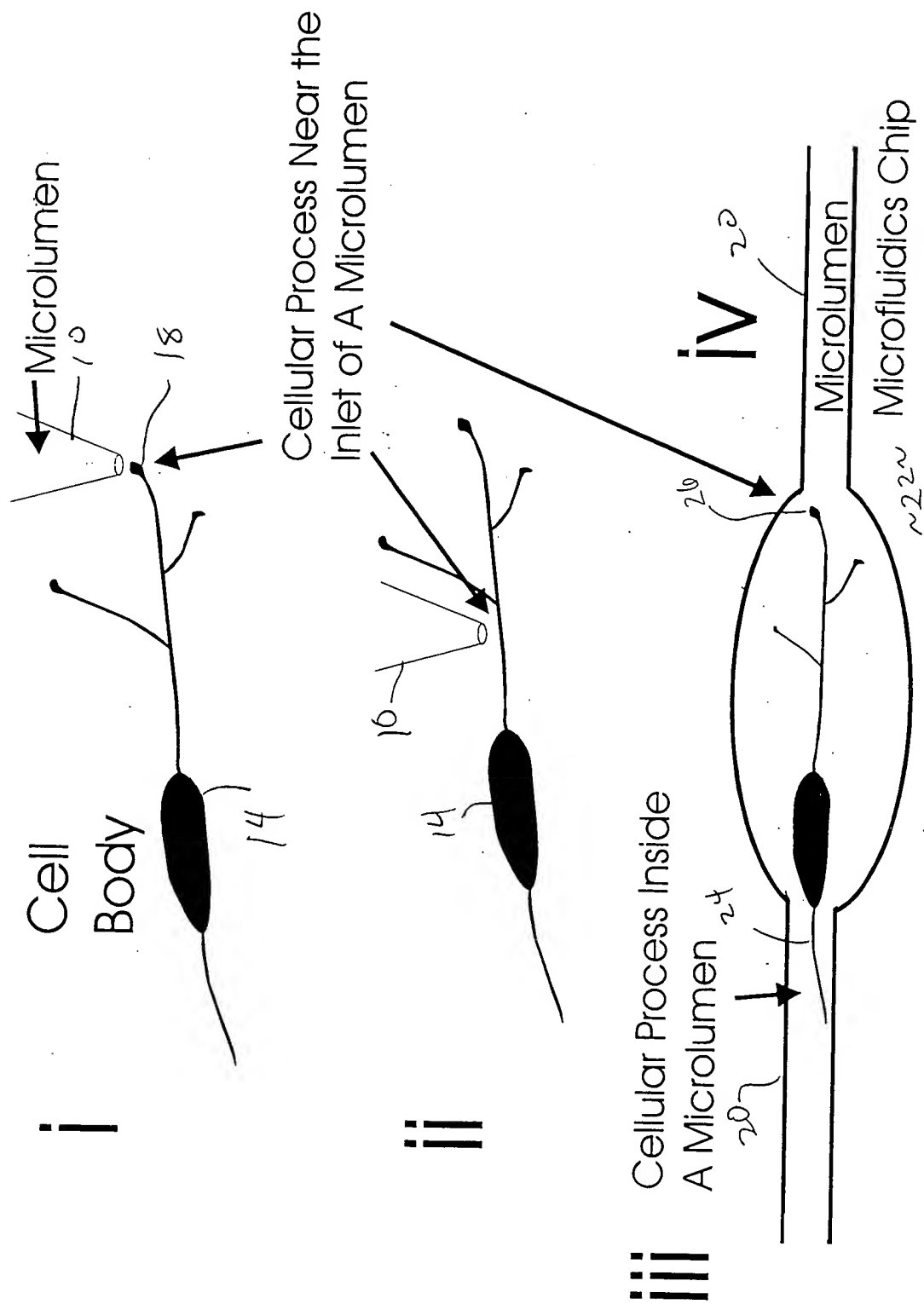


Fig. 6C

## Analyzing A Neuronal Process

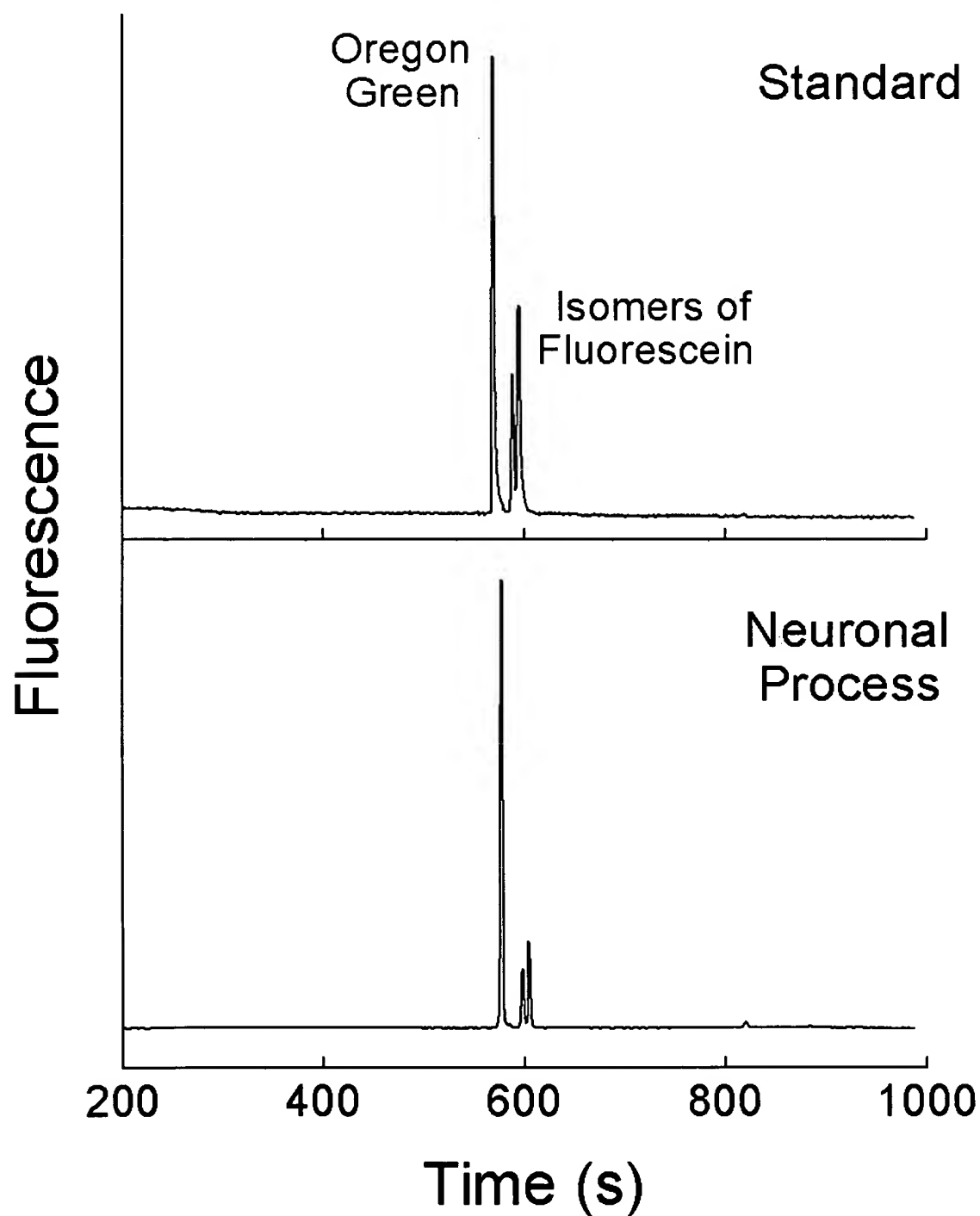
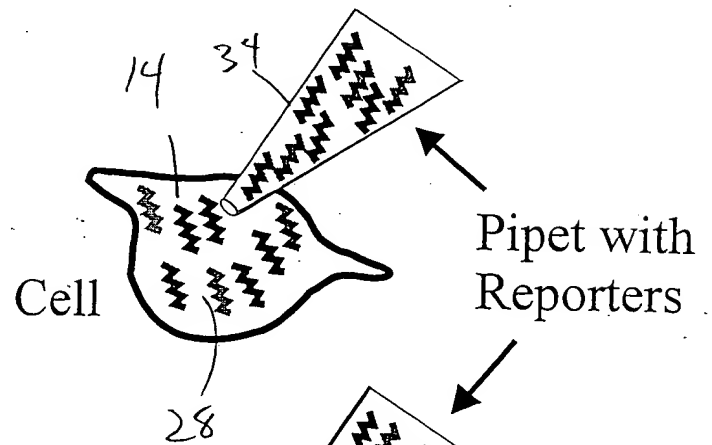


Fig. 8

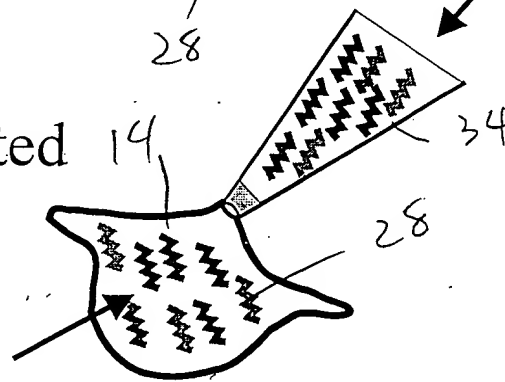
# Loading Single Cells With Enzyme Substrates

Microinjection



Simple Lipid-Assisted  
Microinjection

Reporters  
in the Cell



Optoinjection

Reporters in the  
Solution Around  
the Cell

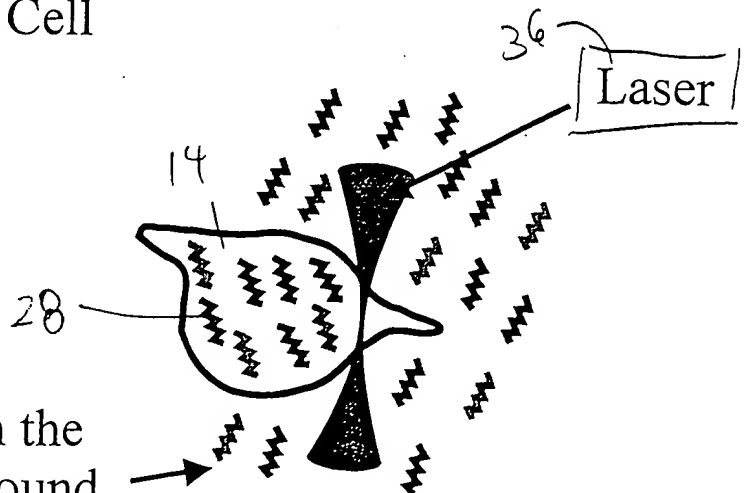
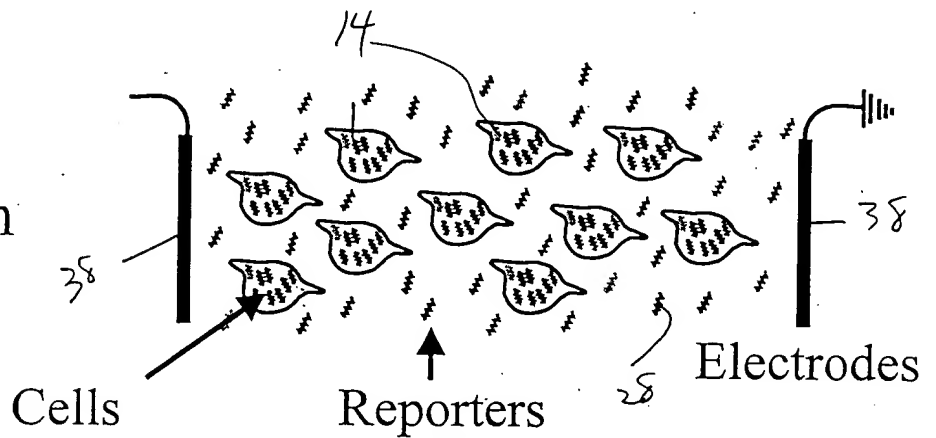


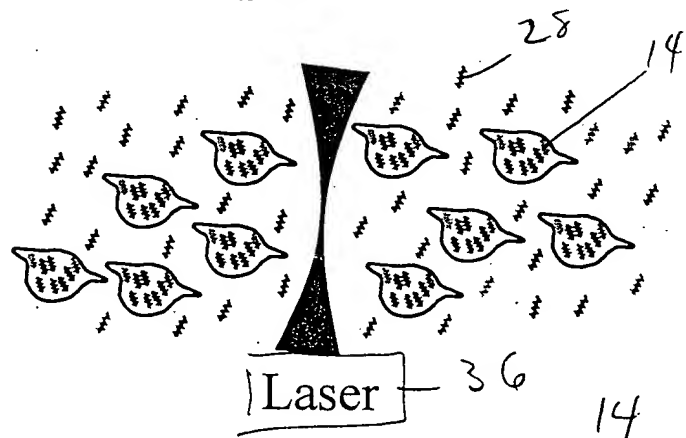
Fig. 9

# Loading Multiple Cells With Enzyme Substrates

Electroporation



Optoporation



Passive Techniques

Pinocytosis

Vesicle Fusion

Membrane-Permeant  
Substrates

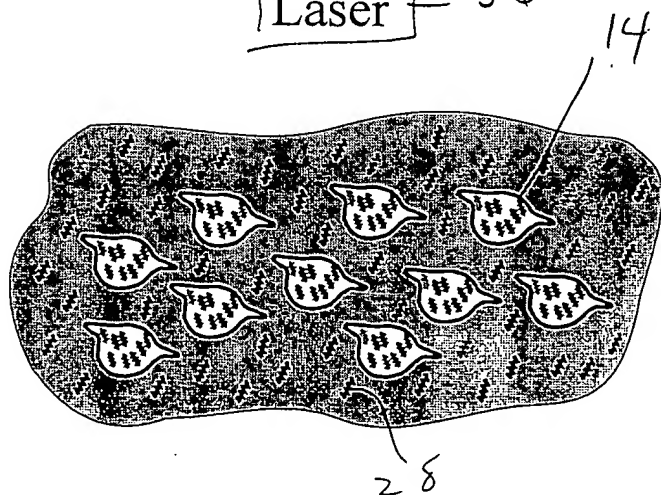
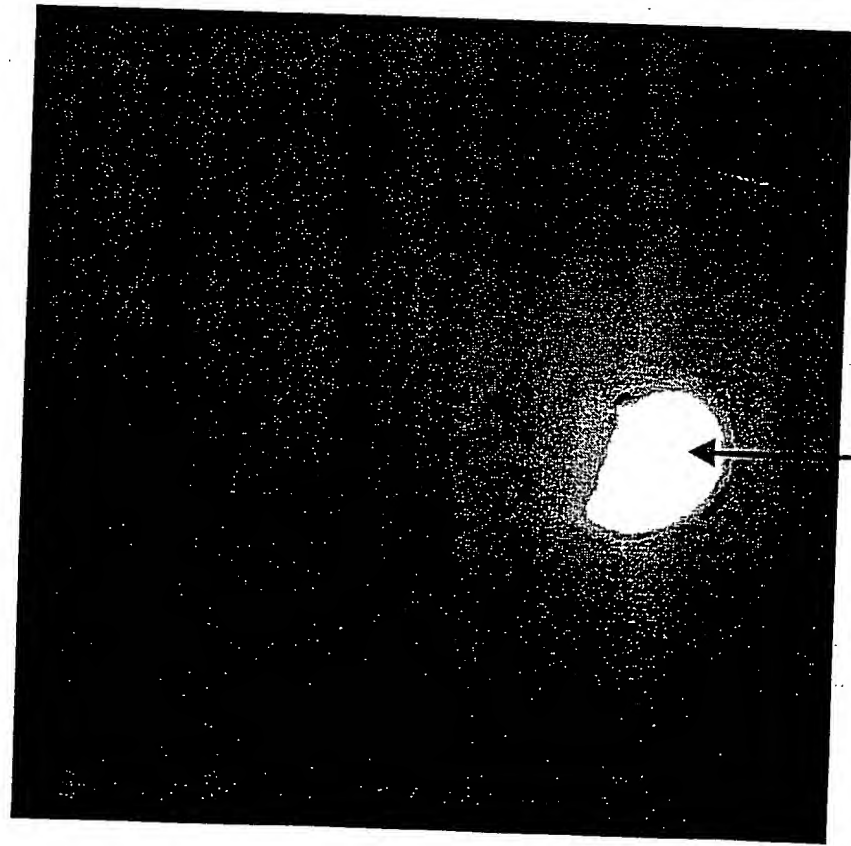


Fig. 10

Nuclear-Localized Substrate for PKC  
Fluorescence Image      Transmitted Light Image



Nucleus of Cell



Cytoplasm of Cell

Fig. 11

# Coupling to Other Technologies

## Proteomics

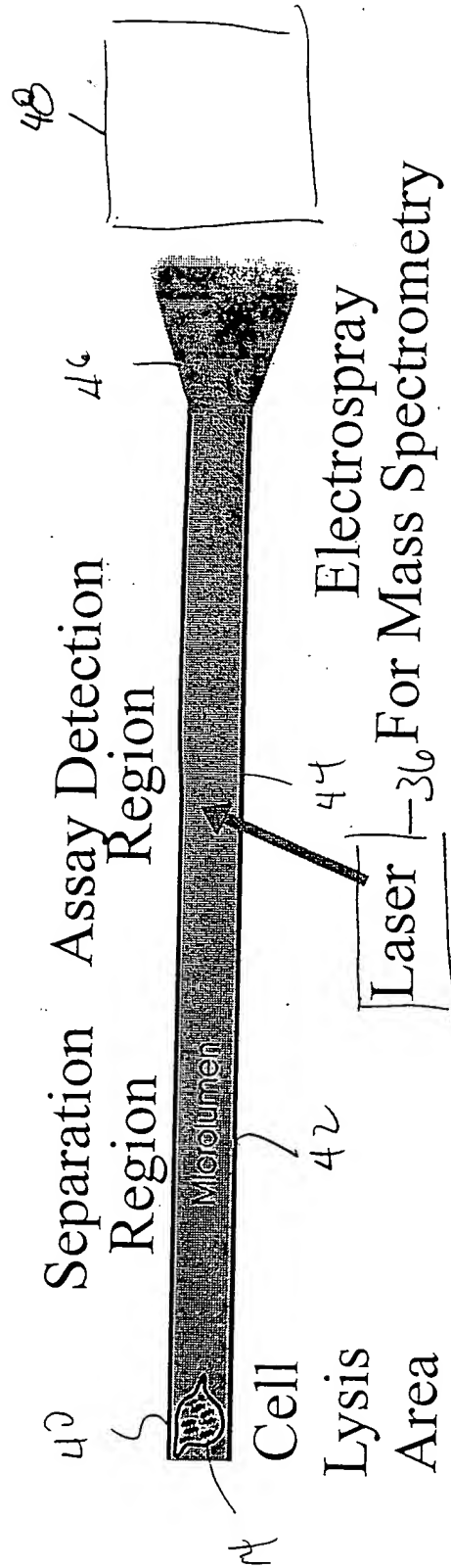
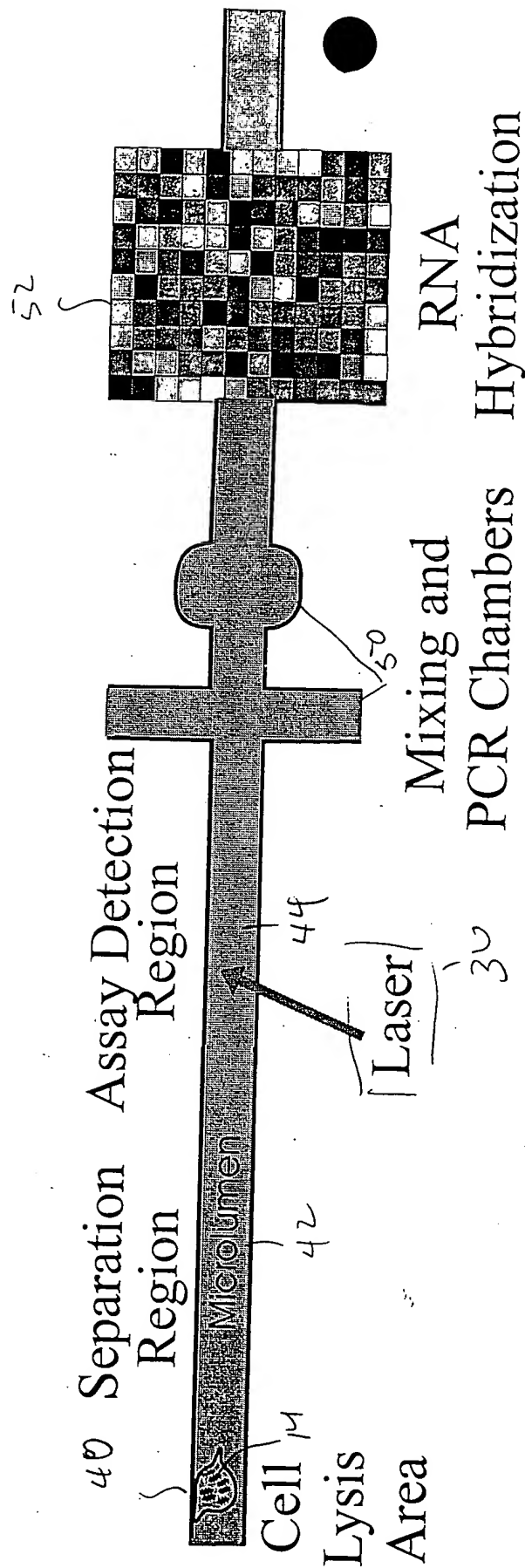


Fig. 12A

# Coupling to Other Technologies

## Genomics



# Fig. 12B

## SIGNAL TRANSDUCTION MICROCHIP

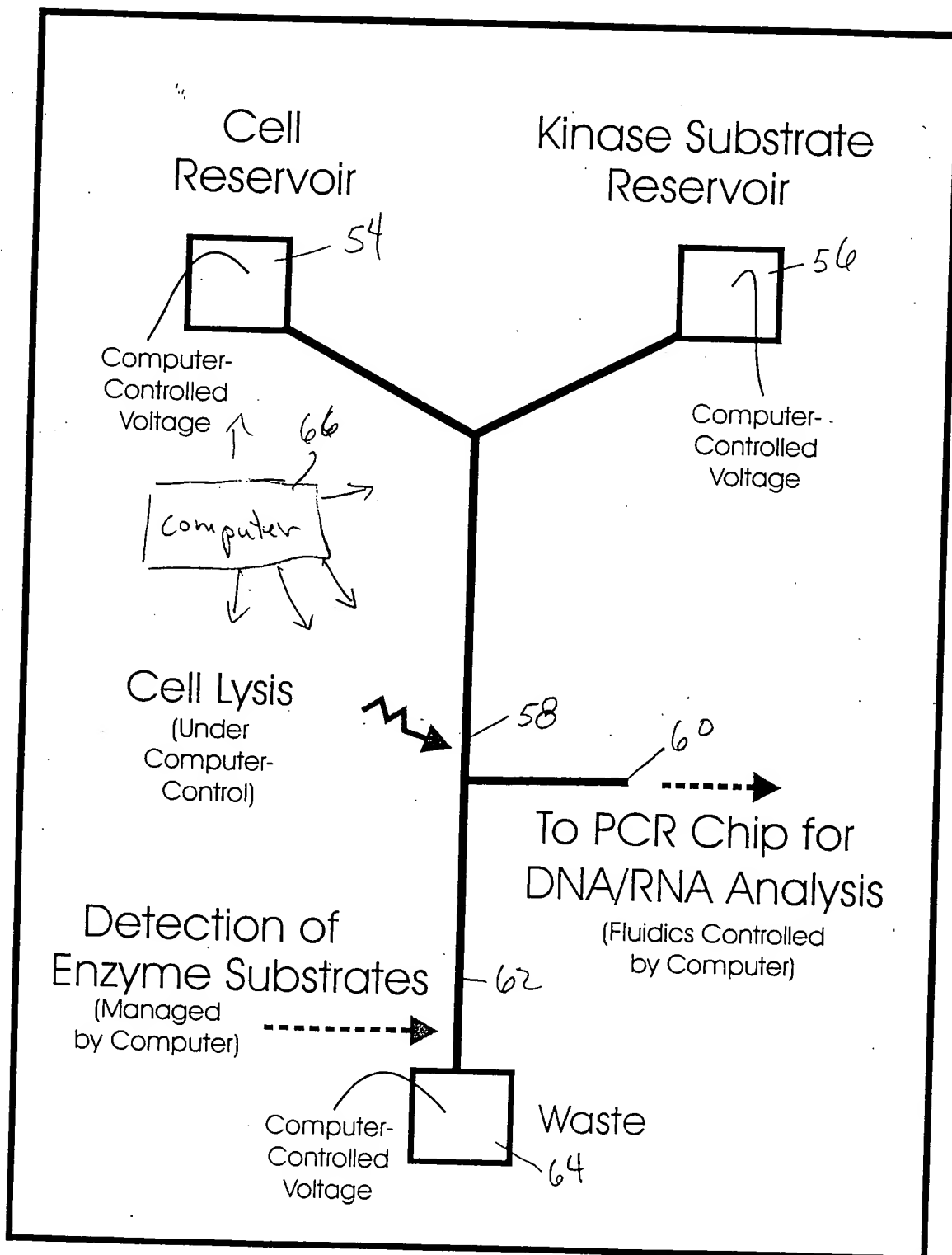




Fig. 13

# Coupling to Other Technologies

## Flow Cytometry

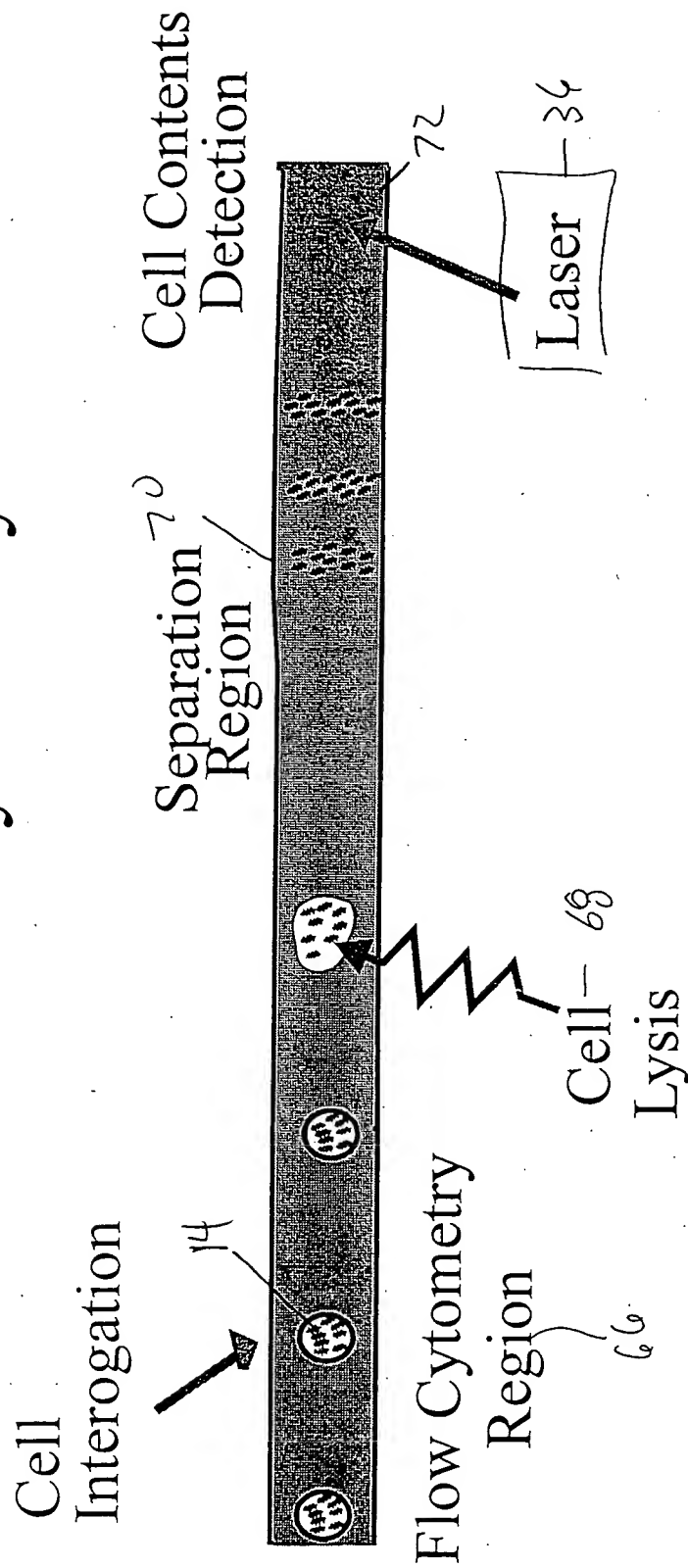
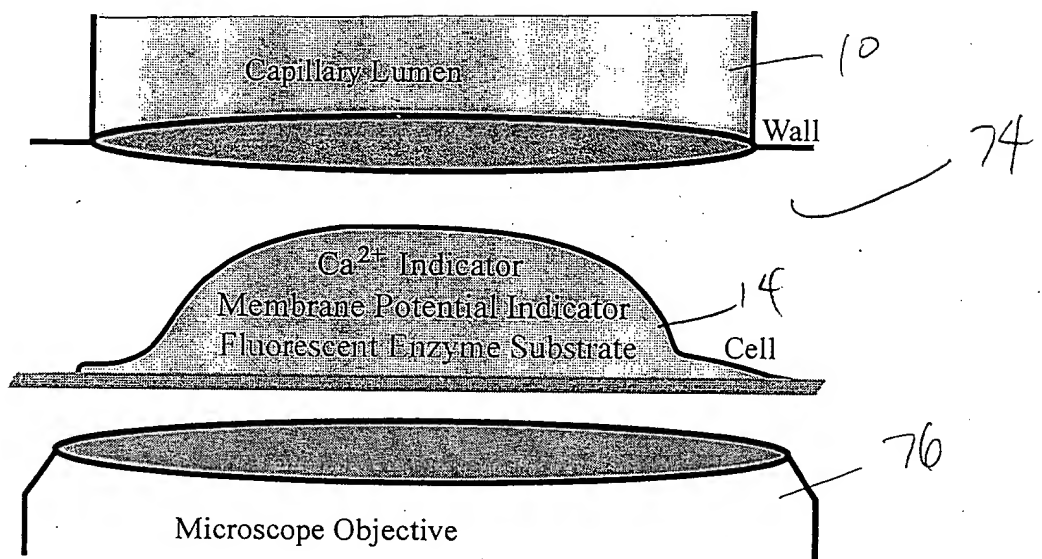


Fig. 14

## Integration With Other Cellular Analysis Methods

### Fluorescence Imaging



### Patch Clamp

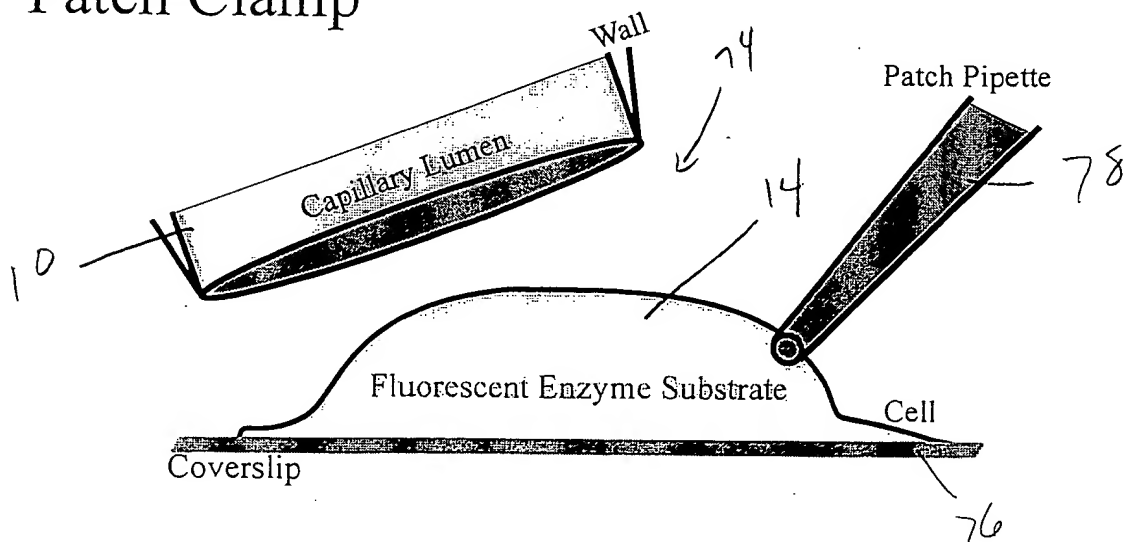
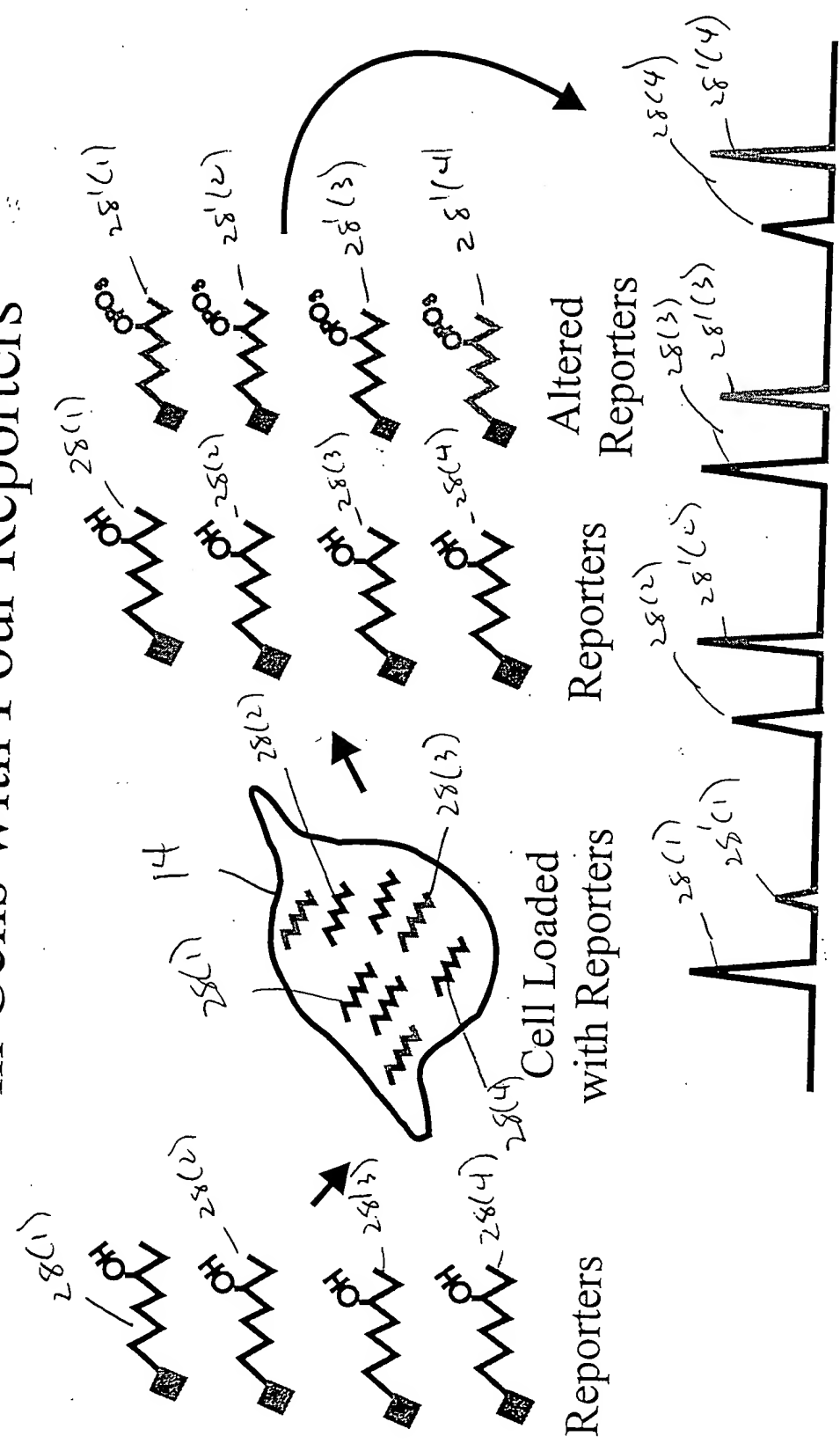


Fig. 15A

# Profiling Signal Transduction Pathways in Cells with Four Reporters



Separation of Reporters and Altered Reporters

Fig. 17

# Profiling Signal Transduction Pathways in Cells with Ten Reporters

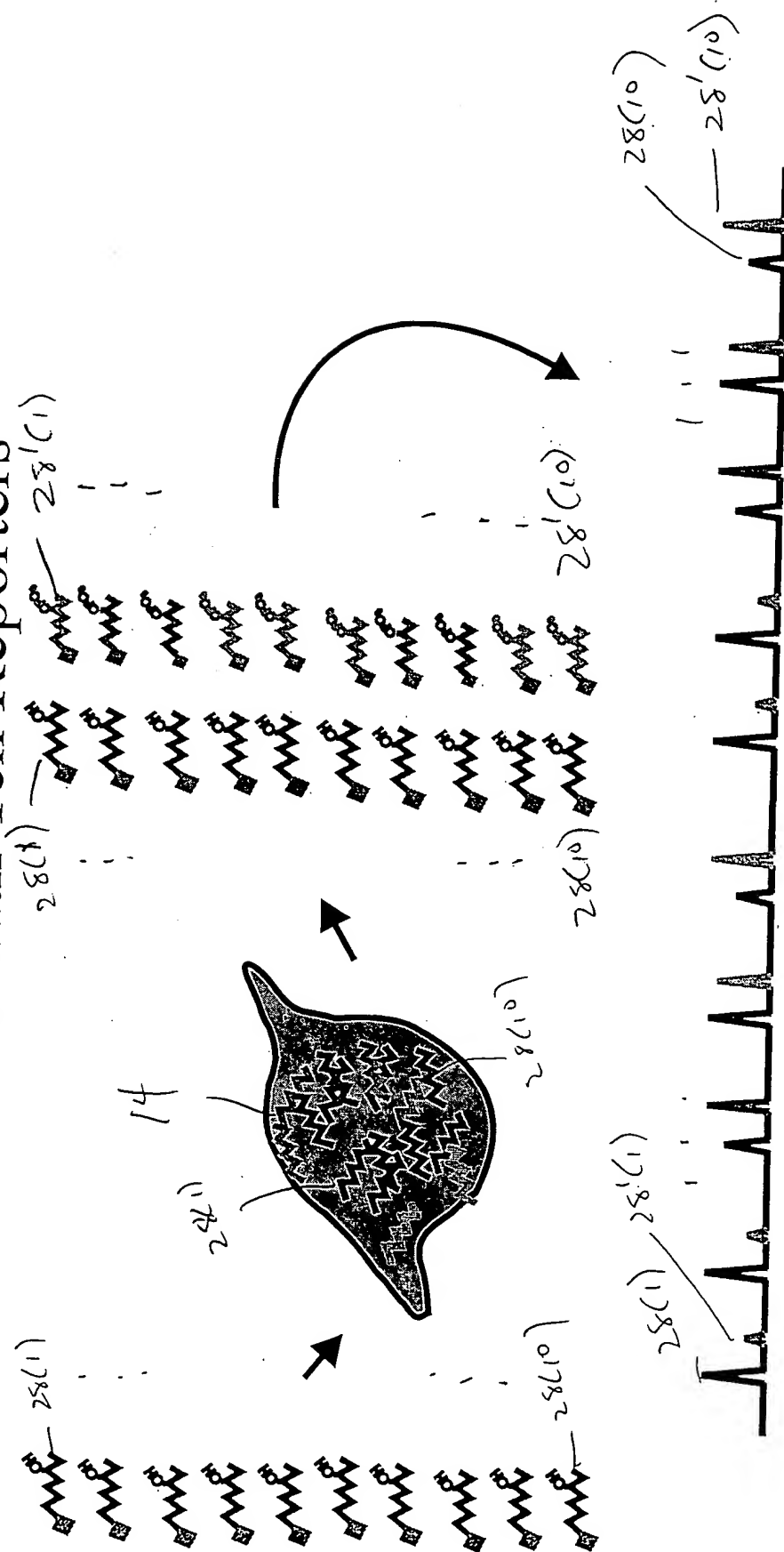
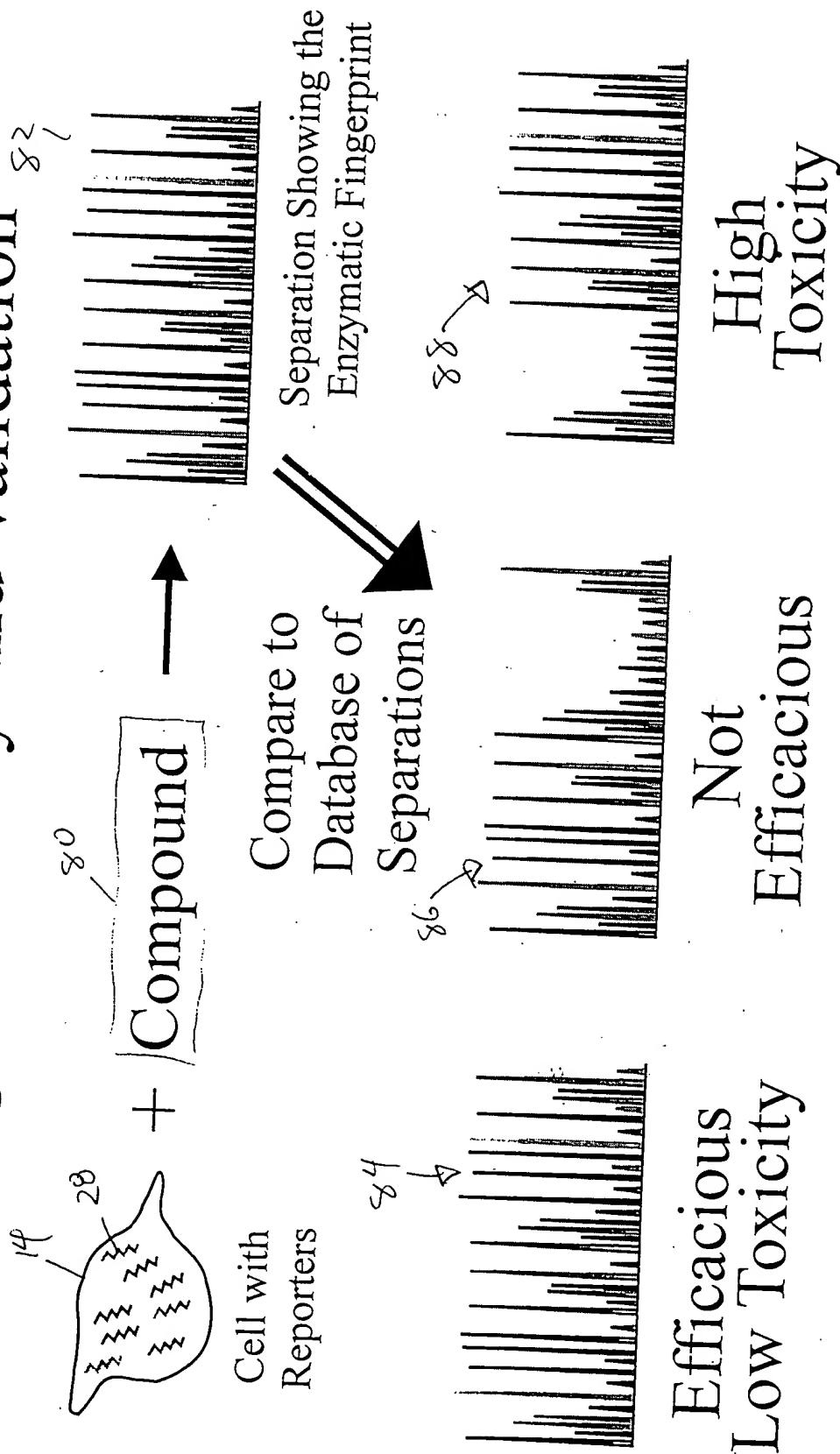


Fig. 19

# Applications

## • Drug Discovery and Validation



# Fig. 20

## Identifying the Cellular Targets of Compounds

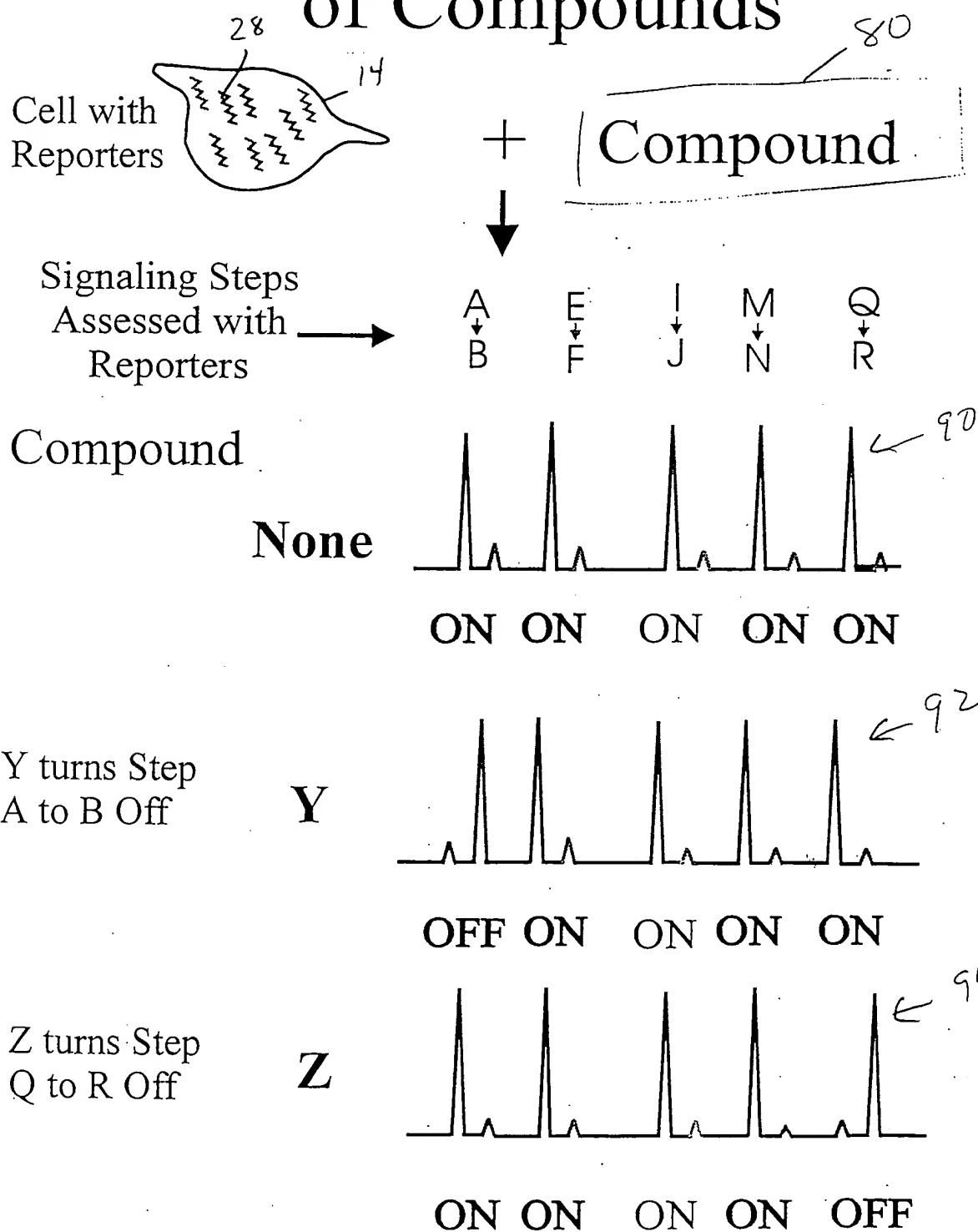


Fig. 21

## Applications

- Diagnostics and Prognostics

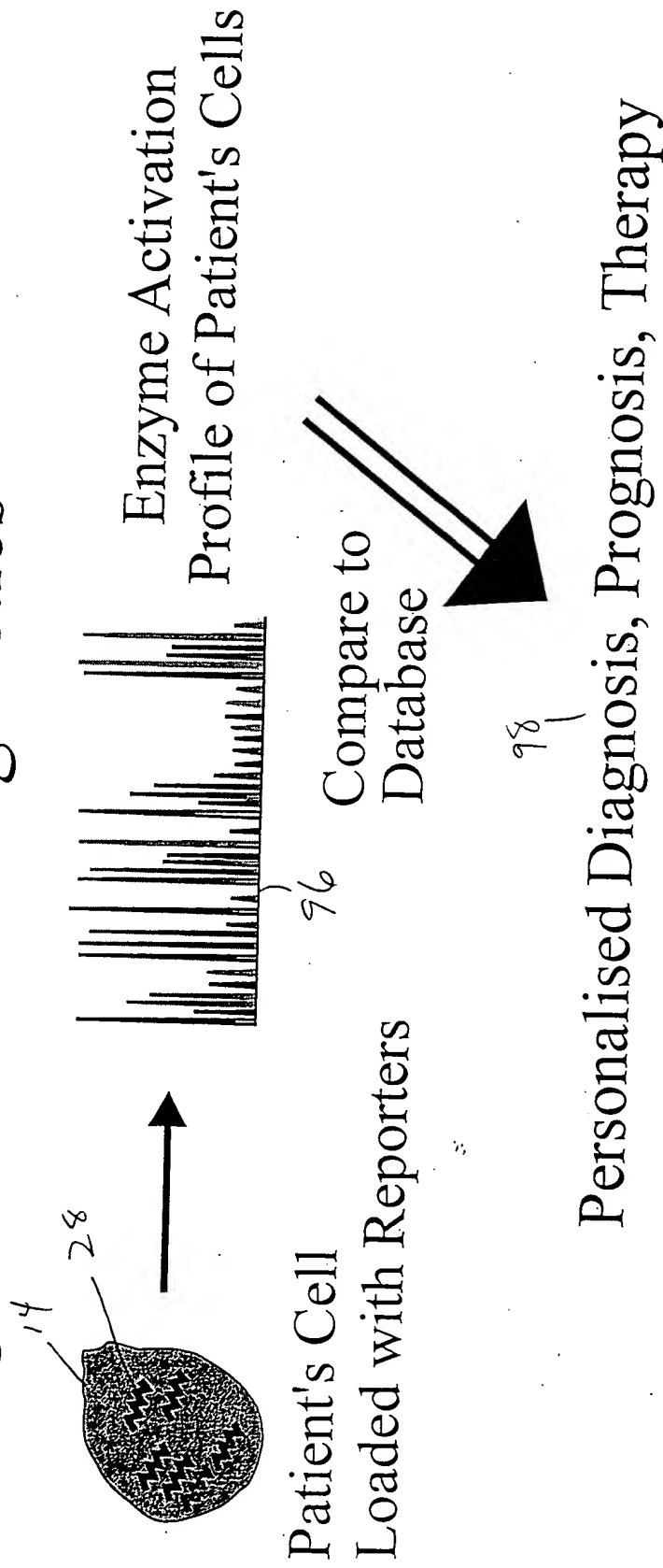
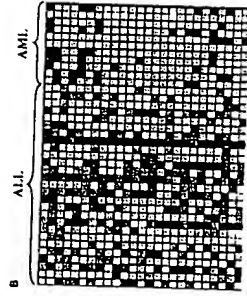
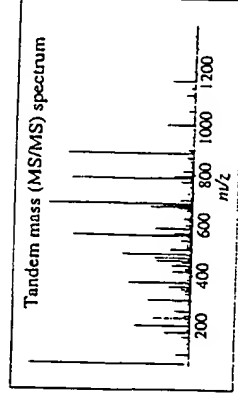


Fig. 23

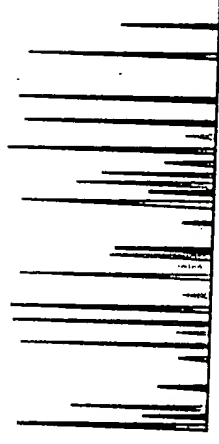
# Analysis of Biologic Systems



DNA Arrays



Mass Spec.,  
Protein Arrays



Protein Activity  
Maps

Genomics

Proteomics

Signaling



Fig. 24

# Serial Analysis of Cells

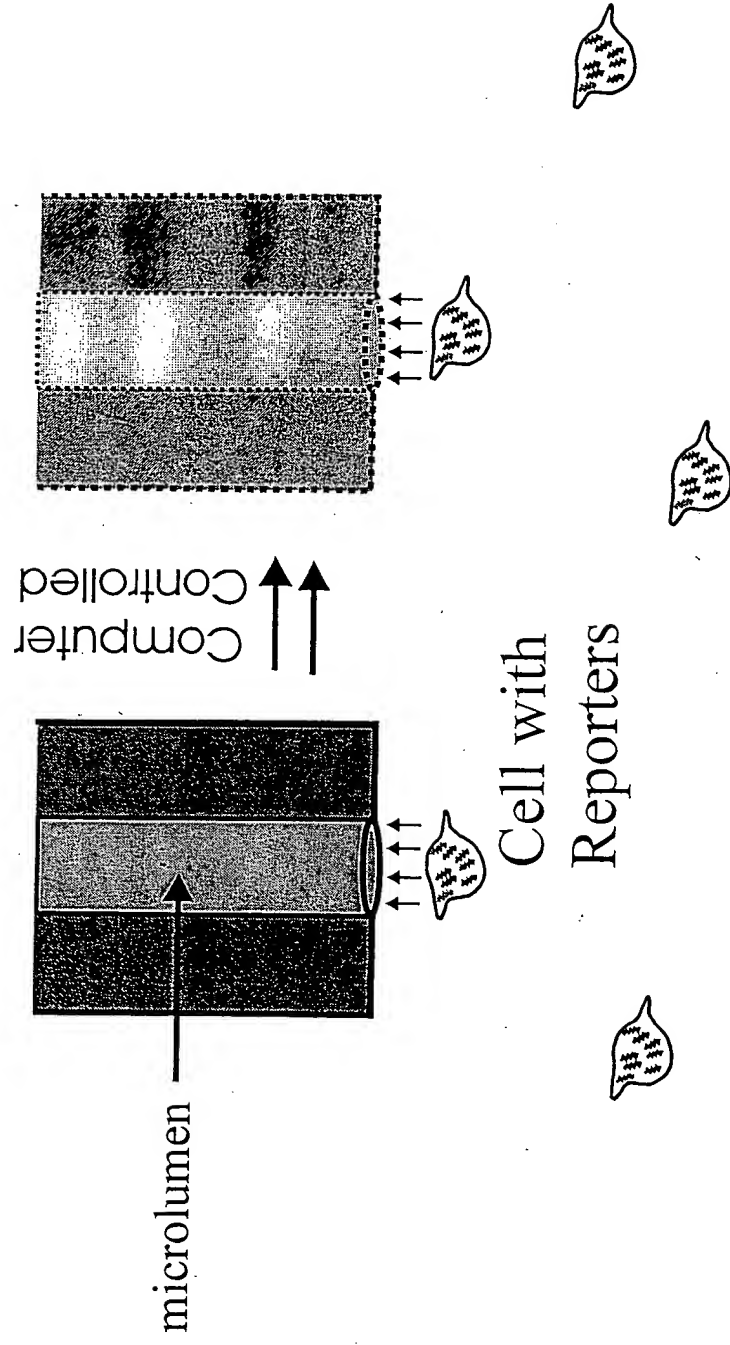
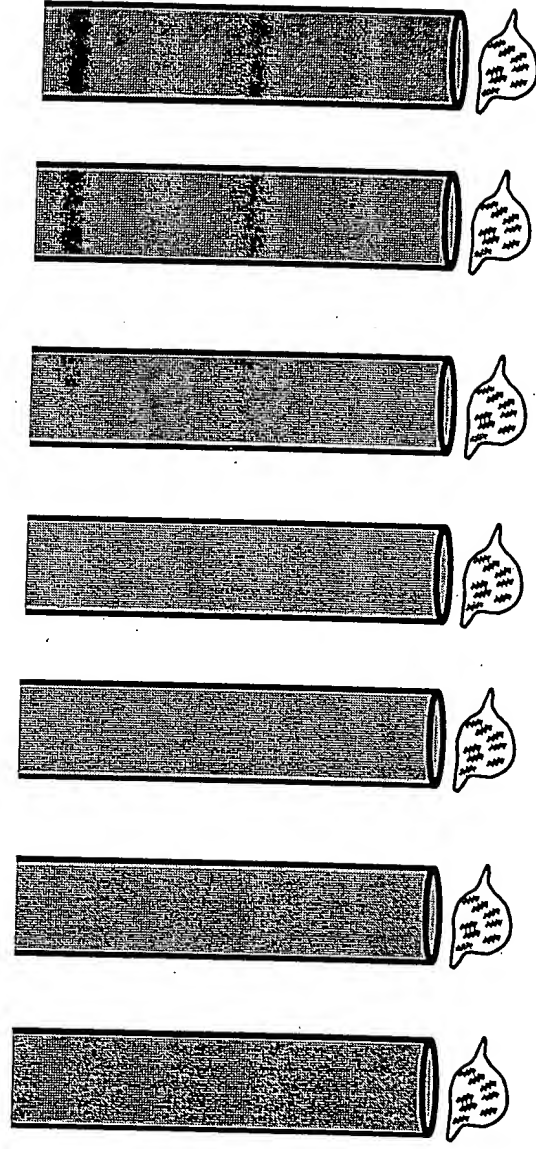


Fig. 25

## Parallel Processing of Cells-

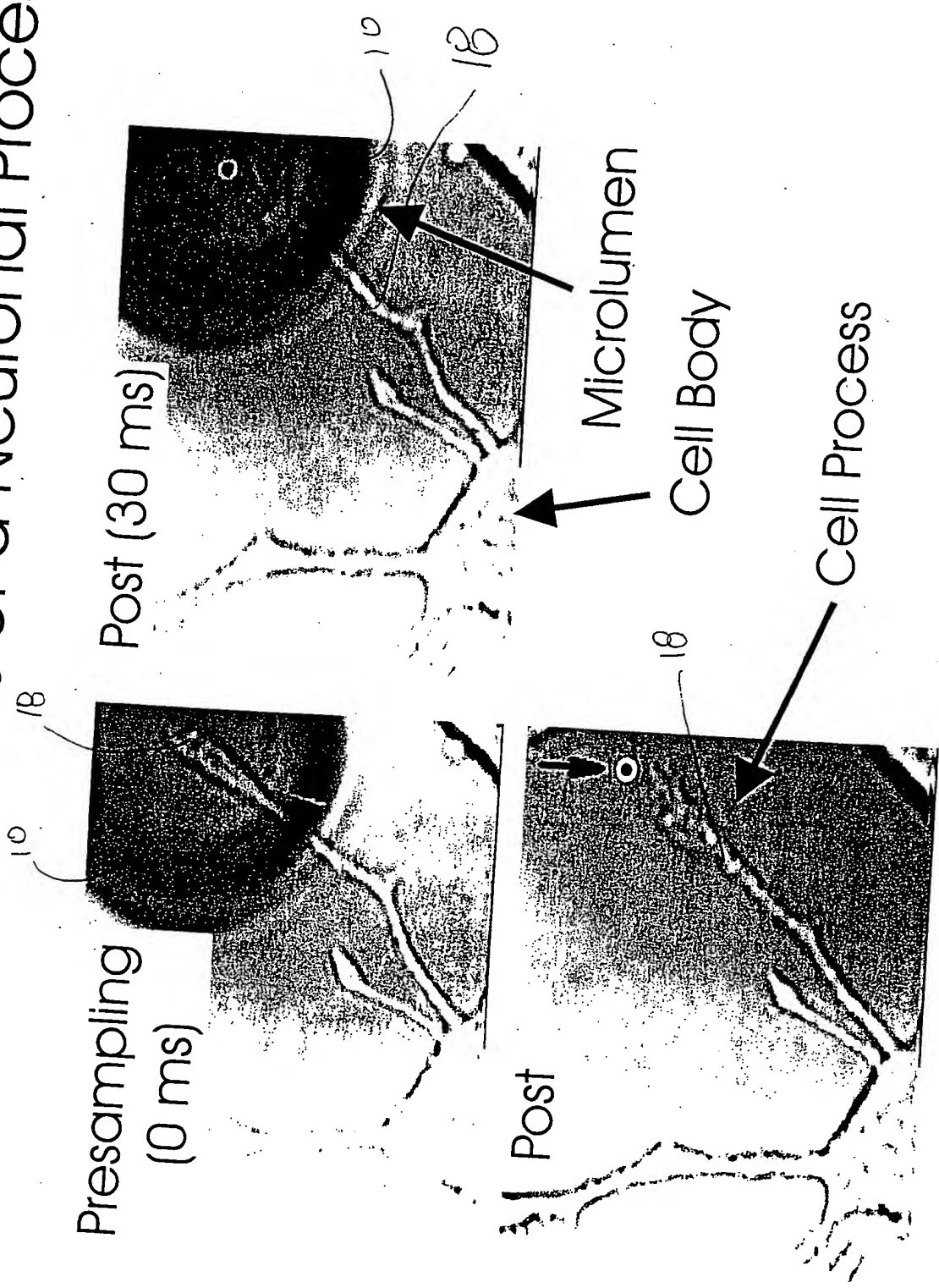
### Arrays of Separation Channels



Computer-control of microlumen alignment  
over cells, lysis, and/or other steps.

Fig. 0B

# Sampling the Contents of a Neuronal Process



1. H.D. 100-100

THE UNIVERSITY OF TEXAS AT AUSTIN

# Fig. 7a Cell Assay

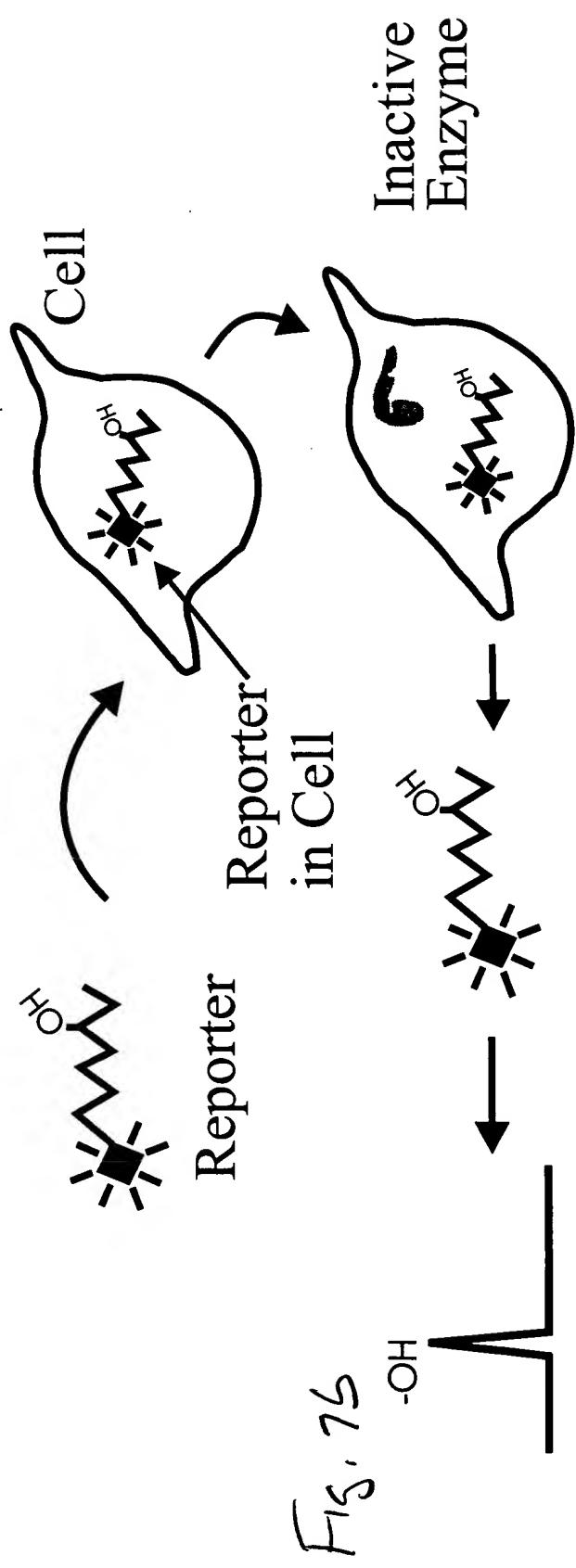
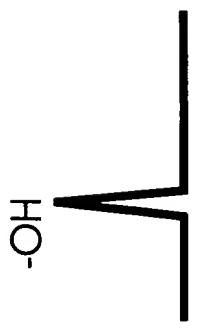


Fig. 7b



OR

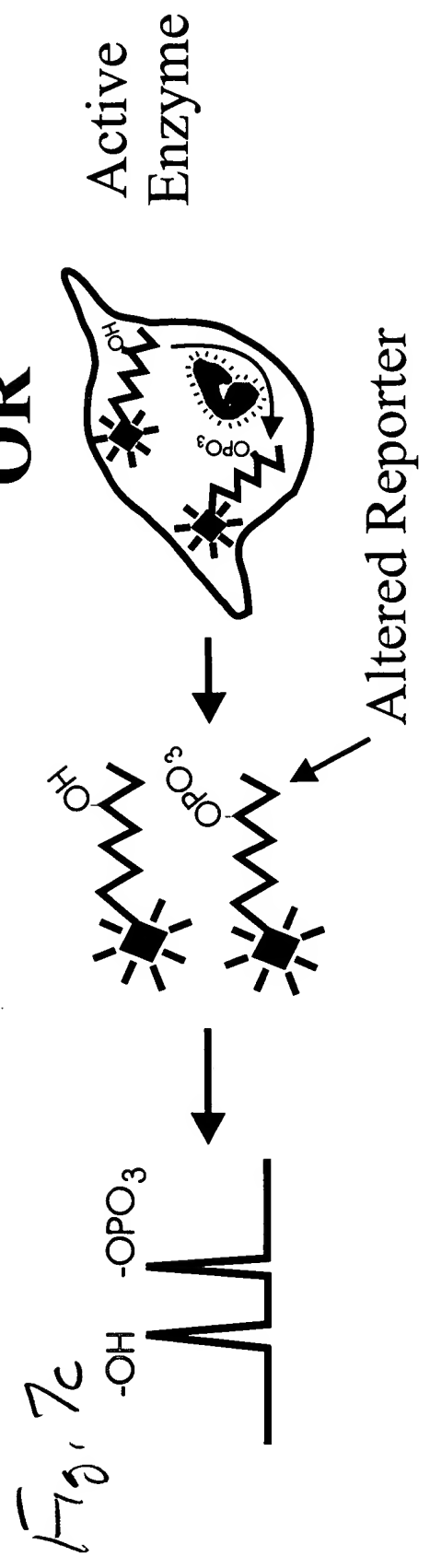
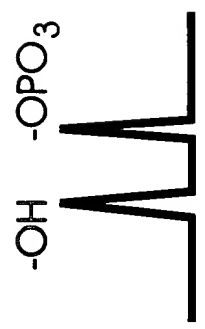


Fig. 7c



# Fig. 15B

## Profiling Signal Transduction Pathways in Cells with Three Reporters

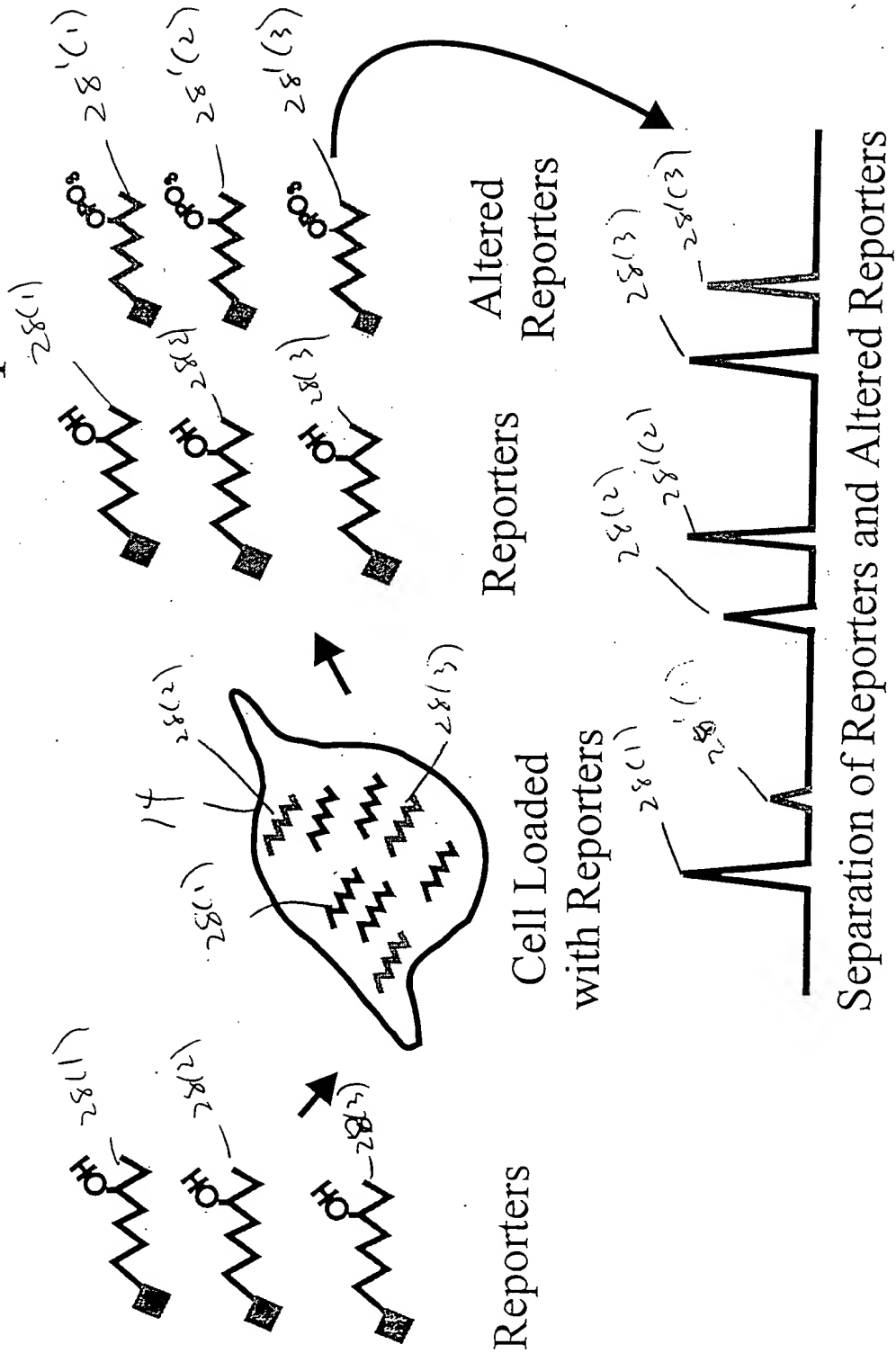


Fig. 16

# Profiling Signal Transduction Pathways in Cells with Five Reporters

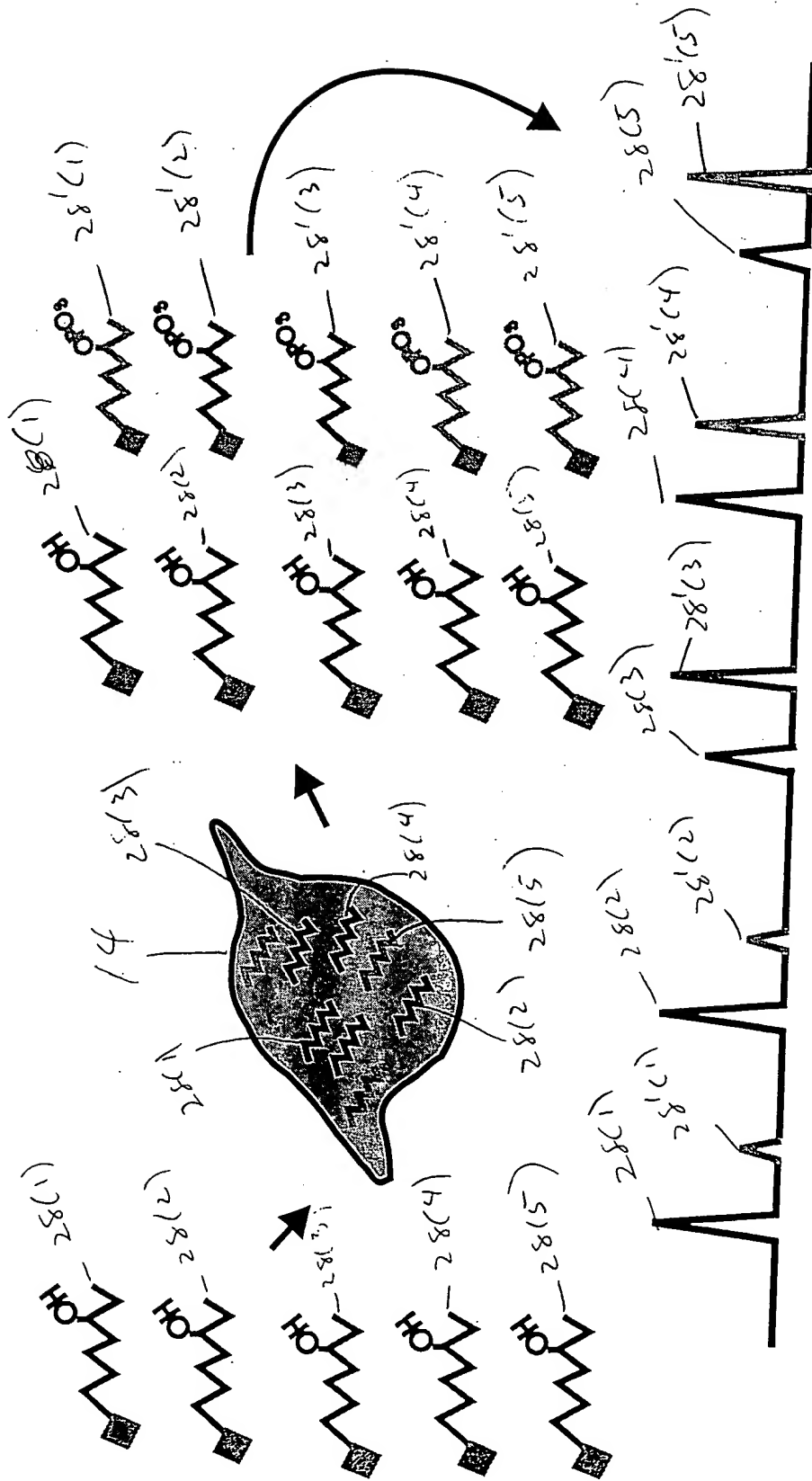
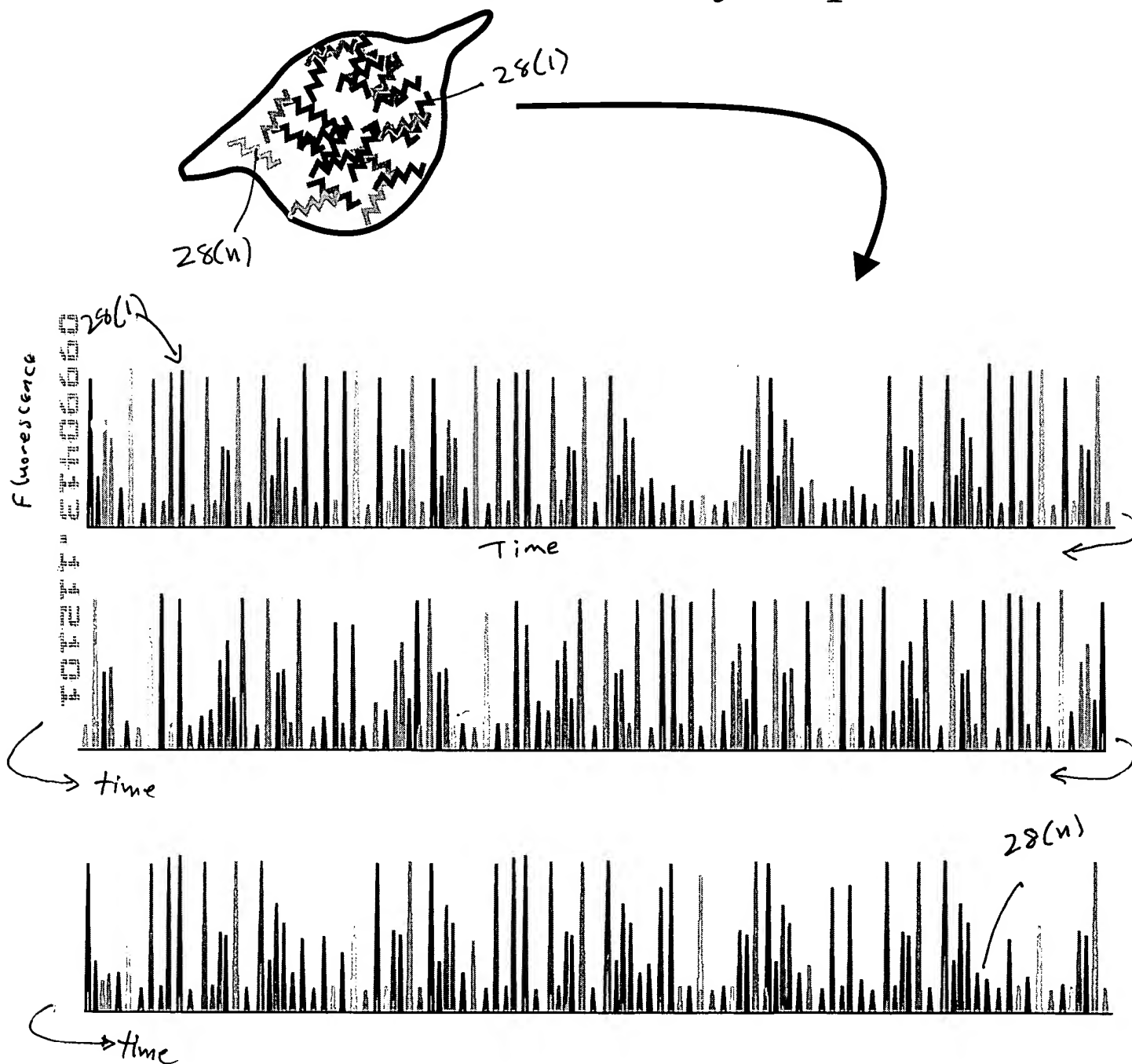


Fig. 18

# Profiling Signal Transduction Pathways in Cells with Many Reporters

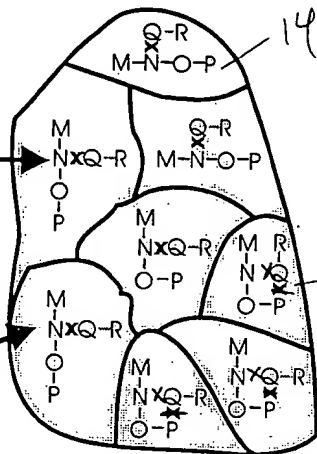


# Fig. 22

## Identifying and Targeting Pre-Disease or Disease States

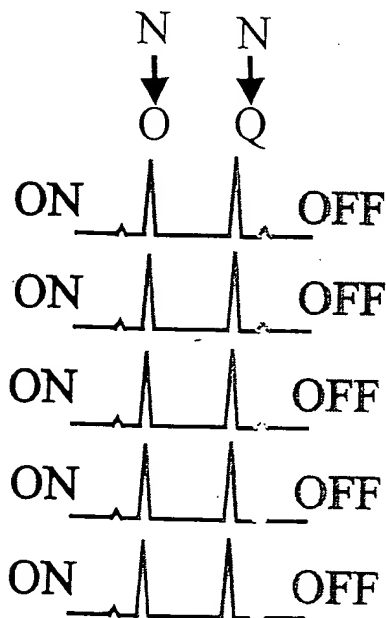
Cells with  
Signaling  
Pathway  
Depicted

Normal Cell  
with N to Q  
inhibited but  
N to O is not  
inhibited

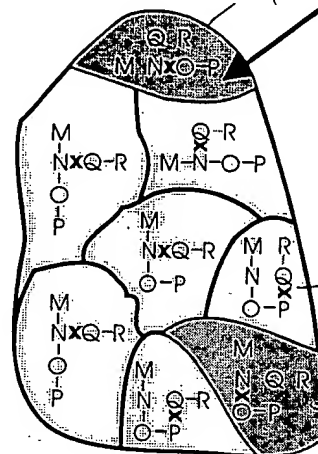


Normal

Analyze ↓



Abnormal Cell  
with N to Q not  
inhibited but  
N to O is  
inhibited



Pre-Disease  
or Disease

Analyze ↓

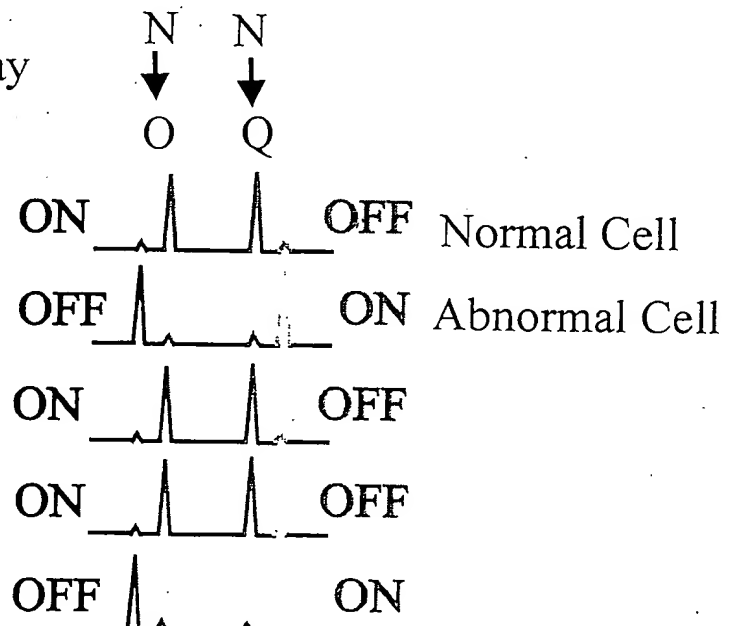




Table I  
Influence of the  
Intracellular Environment

Cellular Property	Is it the same after removal from the cell?
1. DNA, RNA (sequence, quantity)	Yes
2. Protein (identity, conc.)	Likely
3. Activity	Usually Not



# Table III

## A Sampling Of Available Technologies

Field	Property	Technologies
1. Genomics	DNA, RNA	DNA Arrays
2. Proteomics	Protein Identity & Conc.	Protein Gels/Arrays Mass Spec.
3. Signaling	Activity	GFP-Based Methods Critical Need